THE SPERMATOGENESIS OF SYRBULA AND LYCOSA, WITH GENERAL CONSIDERATIONS UPON CHROMOSOME REDUCTION AND THE HETEROCHROMOSOMES.

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The present paper presents observations on the spermatogenesis of *Syrbula* (a grasshopper) and of *Lycosa* (a spider), together with some broader conclusions in regard to questions of the behavior of the chromosomes during the maturation mitoses, and of the nature of those modified nuclear elements which have been termed by me heterochromosomes.

The subject of heredity, which in its broadest sense includes most of the problems of the phenomena of life, is being pursued from two main lines: from that of the study of the germ cells, and from that of an analysis of the results of cross breeding. The actual steps of the process of heredity, if we shall ever understand them, will be learned by the first method, by the investigation of the energies of those cells which transmit ancestral traits. The second method is of less importance than the first, for while it may permit an analysis of the proportional transmission of different ancestral traits, it can in no way elucidate the steps of this process, for the very reason that its material basis is the terminal stage alone, and the somatic condition at that.

And of all cellular investigations, those that concern themselves with the nuclear chromosomes seem to penetrate deepest into the mysteries of the problem, for these cellular components more than all others seem proven to be the centres of hereditary energies; can we unlock their secrets we will have opened the door to the light, for there seems to be no other portal.

The study of the chromosomes has ceased to be regarded as an academic question, or as a mere side issue of problems of cell division, and is slowly but surely coming into the centre of the field of biological thought—of that thought which embraces the broader community of natural phenomena and does not lose sight of the forest for the trees. All things are in the nature of processes, to the biologist of genetic processes, and of the vital changes heredity is the one that is the most comprehensive; broadly speaking, biology is the study of heredity. There are two methods used in the search for the solution,

the morphological and the experimental. The former reasons out the process as it is to be understood from the sequence in structural change; the latter aims directly at an analysis of the process by a study of result where the cause is measurable. Yet just here it must be held in mind that the true morphologist has in ultimate consideration the explanation of process, so that he is fully as much a physiologist as the other. And his method is correct, because structure only is visible while process is an intangible change, and therefore he is reasoning from the perceptible to the imperceptible. Many morphologists do not conceive this mental attitude rightly, and most physiologists are inclined to hold that all morphologists see no further than the structure. Yet the morphological basis must precede the physiological experiment, and it is quite questionable whether both will not always be necessary as complemental methods; we cannot say which will ultimately prove the more important, but all will admit that the greater interpretations of biology have had a morphological basis, and that the morphologist has done his full half in reasoning out the processes.

That is not scientific morphology which goes no further than the structural fact; but with minor exceptions all morphologists try to go much further than this, and throughout their analyses have in mind the process. And the morphologist is an analyst of natural phenomena, an explainer of those normal experiments not performed within the laboratory. Therefore a present tendency to maintain that only experiment can furnish explanations, and that structural study can present only observational results, has no foundation whatsoever. The true method is to remember always that in the living as in the non-living world the process must be interpreted; so long as this is not forgotten it matters little what mould the investigation is cast in.

Some years of rather intensive study of the structure of the germ cells, particularly of the behavior of their chromosomes, has led me to the conclusion that there is simplicity and essential uniformity among the bewildering maze of the observable. When we strive to explain the more complex from the more simple we discover this uniformity, but not when we stubbornly persist in regarding the more complex as the condition that can be immediately explained. Complete agreement of opinion there may never be, but this is because of mental differences and not of lack of uniformity in the natural processes. A main reason for the failure to interpret the uniformity has come from one of three preconceptions: of persistent study of an object which has shown itself incapable of furnishing a clear solution; consequently of the neglect of seeking comparative evidence; and of loyalty to the views

of the first workers in the field, or fear of conflict with them. In common law a man is held innocent until he is proven guilty, but in scientific thought we should consider a view erroneous until it is proven to be correct to fact. That view which presents phenomena from the simplest interpretation, which is based upon the broadest comparative series of facts, and, above all, which admits of no exceptions in natural sequence, is the one which in the end has the greatest probability of maintaining itself, because the one most likely to be congruous with the facts.

1.—Spermatogenesis of Syrbula acuticornis Bruner.

Testes of adults of this Acridid were collected at Austin, Texas, in the middle of October, fixed in Flemming's stronger fluid, and stained with iron hæmatoxyline. For the identification of the species I am indebted to Mr. James A. G. Rehn, of the Academy of Natural Sciences, Philadelphia. A considerable number of testes were sectioned and studied, whence it resulted that some of them contained ten bivalent chromosomes in the first spermatocytes, others twelve. I cannot determine whether this is due to *Syrbula acuticornis* being a form including more than one species, or whether it is a single species with individual variation in the number of the chromosomes; the latter alternative would be in contradiction to the condition maintaining in most species. Because this point could not be explained, and because good proof is necessary to establish the occurrence of individual variation in the number of chromosomes, the following description is limited to cells contained in the testes of one individual.

Work has been done previously upon the spermatogenesis of Acrididæ by Wilcox (1895), McClung (1900) and Sutton (1900, 1902). My results are in essential agreement with those of McClung, except with regard to the time of the reduction division. Carnoy (1885) was the first to describe cell divisions of male germ cells in Acrididæ, and figured in detail spermatogonial mitoses.

As in the Hemiptera each testis is composed of long tubular follicles, but they are more numerous in number, the earlier stages of the sperm cells placed at the proximal end of the follicle, and the later stages at successively following regions of the follicle. As far as I have noticed there is no difference between the cells of different follicles, beyond a dimensional one.

Two generations of spermatogonia are found in the mature testis, the smaller of which is the last generation, and by division forms the first spermatocytes. The intermediate body or cell-plate (*Zwischen-*

körper of the German writers) persists for a long while after the division of the penultimate generation of spermatogonia, even up to the prophases of the last spermatogonic mitosis. Accordingly the rest stage of the last generation of spermatogonia (Plate IX, fig. 1) shows a distinct polarity of the cell-body, with a distal pole at which is the persisting cell-plate, and a dark mass of idiozome substance, which appears to be in part, at least, derived from the connective fibrils of the preceding mitosis; and an opposite or central pole containing the nucleus. The nucleus shows minute chromatin globules distributed in bead-like chains along the linin fibrils, and also accumulated in larger masses. With great regularity there is found also in each nucleus two or three larger, somewhat irregular, deep-staining bodies; whether they are nucleoli or heterochromosomes could not be decided by the use of the iron hæmatoxyline stain.

The prophases of the last and penultimate spermatogonic mitoses appear similar in character. The chromatin seems to arrange itself into a continuous spirem, or, if not into one thread, certainly into but a small number of very long threads. Plate IX, figs. 2–6 illustrate a succession of the later prophases, and all show stages of segmentation of the spirem. Fig. 2 shows a pair of minute centrosomes just external to the idiozome body, and figs. 3 and 5 successive stages of the central spindle; the nuclear membrane commences to dissolve first in the vicinity of the central spindle. The only point deserving particular comment in the stages is a chromatin element, marked *N. 2* in the figures, that is found in every cell; it is a portion of the chromatin spirem of smaller diameter than the other segments, much more convoluted and in such a manner as to represent a small corkscrew, and frequently appearing to be enclosed within its peculiar membrane. It resembles in this respect the accessory chromosome described by Sutton (1900) for the spermatogonia of *Brachystola*. It is an element that appears to be retarded in its stages on comparison with the others—not condensing nor segmenting as rapidly as they do. When the nuclear membrane has completely dissolved away this single loop segments into two, which are still to be distinguished from other chromosomes of the same length by narrower diameter and more spiral form. These two chromosomes resulting from the division of the single convoluted element are probably the heterochromosomes which become much better demonstrable in the spermatocytes; for the heterochromosomes of the spermatocytes differ from the other chromosomes in their behavior, as will be shown, and this pair in the spermatogonia behave at first differently from the others. Because these heterochromosomes are demonstrable in such

early spermatogonic prophases, we can conclude that they must be present in the rest stage of the nucleus, though merely in the form of constituents of the chromatin reticulum. And their juxtaposition in the chromatin spirem is a point in evidence of an earlier contention of mine (1900, 1904*a*), that in the chromatin spirem of spermatogonia homologous chromosomes, *i.e.*, such as unite into pairs during the consequent synapsis stage, lie next each other. All the chromosomes become longitudinally split during the prophases.

Two clear pole views were found of the spermatogonic monaster stage (metaphase), Plate IX, figs. 7 and 8. Each showed exactly twenty chromosomes. These occur in pairs, and we can distinguish three largest pairs (*A, a; B, b; C, c*) and three smallest (in succession from the largest to the smallest, *F, f; D. d; E, e*). The exact similarity in form and size of the members of a pair does not evince itself so clearly in a camera drawing as in the study of the chromosomes themselves, because the members of a pair usually do not lie exactly in the same plane. So twelve of the twenty chromosomes can be demonstrated to form six pairs; the remaining eight chromosomes are so nearly of the same size and form that their arrangement into pairs cannot be shown, but by analogy with the others it is probable they constitute a series of four pairs. One pair of the latter four probably corresponds to the pair of heterochromosomes found in the prophases, but their earlier peculiarity of convoluted shape no longer persists, so they offer no means for recognition. The spermatogonia, accordingly, contain each two heterochromosomes and eighteen ordinary chromosomes.

All these chromatin elements were longitudinally split, and became so placed upon the spindle (Plate IX, fig. 9) that the daughter chromosomes separate along the line of this split; fig. 10 shows an early anaphase. Fig. 11 is a pole view of one of the two first spermatocytes resulting from this division, and shows exactly twenty chromosomes. Therefore the first spermatocyte receives a half of each of the two heterochromosomes and of each of the eighteen ordinary chromosomes. In each first spermatocyte, daughter cell of the last spermatogonic division, the nucleus commences to reconstitute itself (fig. 12). The nuclear membrane reasserts itself, the chromosomes commence to elongate and take on more irregular contours; but an interesting phenomenon is that two of the chromosomes (*n. 2*, fig. 13) do not undergo these changes, but remain smooth and dense; these are heterochromosomes, and in all probability identical with those in the spermatogonia. At a later stage (fig. 14) these unite to form a single bivalent heterochromosome (*n. 2*), and they retain this condition up to the time of the first

maturation mitosis. The other chromosomes have become long and thread-like, and an irregular nucleolus (*N.*) has appeared. Following the stages of figs. 14 and 15 is reached a complete rest stage (fig. 16), with the chromatin globules finely distributed along the linin threads—the nucleus very similar in appearance to that of spermatogonia in the rest stage, except for the presence of the large heterochromosome. A rest stage preceding the synapsis I have never before found in any object, but it has been described for *Ascaris* and certain other forms. The heterochromosome is still nearly straight, and when viewed from the proper angle shows not only a transverse constriction, marking the point of junction of the two univalent ones, but also a longitudinal split in each of the latter (figs. 15, 16). In later stages of the spermatocyte these characteristics of the heterochromosome cannot be distinguished, and from a study of the later stages alone one might easily be misled to the conclusion that the heterochromosome of the spermatocyte were a univalent element.

Next the chromatin reticulum segregates into short loops, very much convoluted and occasionally simulating longitudinal splittings (Plate IX, figs. 17, 18). But a long study of cells in this period shows that the space between two mutually wound loops is not a longitudinal split, and that the latter, *i.e.*, a splitting into two of each chromatin globule along the length of a loop, rarely ever commences so early. On the contrary the double loops represent pairs of univalent and correspondent (homologous) chromosomes, so that this stage is the commencement of the conjugation into pairs of the eighteen ordinary chromosomes; this becomes the more obvious on comparison with subsequent conditions. Now, also, the heterochromosome commences to bend at an angle at its middle point, on its path from the earlier straight form to its later one of a nearly closed V.

This leads, the chromosomes becoming much longer (Plate IX, fig. 19), to the synapsis stage (figs. 20–22); throughout this stage the nuclear membrane is almost or quite imperceptible, and the chromatin loops in the form of irregular U's and V's, crowded most densely at that nuclear pole (the distal) next the greatest amount of cytoplasm. In all the drawings only a few of these loops are shown, mainly those seen distinctly for their entire length. Their relative lengths differ greatly in size, as is to be seen particularly in fig. 21. Each such loop is a bivalent chromosome, for they are nine in number, corresponding to the nine pairs of ordinary chromosomes of the spermatogonia, and therefore each arm of one is a univalent chromosome. Two univalent chromosomes are usually united only by one end, that marked *x* in fig. 21; but

sometimes the opposite ends also are joined, elongated rings resulting instead of other forms. The space between the two arms of such a bivalent chromosome does not represent a longitudinal split, but the area between two entire bivalent chromosomes. The true longitudinal split becomes apparent as a cleavage of the small chromatin masses forming each univalent chromosome, and is a line of chromatin separation within each univalent chromosome; sometimes it cannot be seen, which is due to the chromosome being viewed from the edge. This is the first and only longitudinal split of the chromosomes from the time of first formation of the spermatocytes up to the stage of the spermatid. No trace of a longitudinal split can longer be seen in the heterochromosome, which now has in most cases the form of two nearly parallel rods, produced by the bending at the middle of the original straight one. This synapsis stage corresponds to the similarly named stage of the Hemiptera in the close massing of the chromosomes near one pole of the nucleus, but we have seen that the conjugation of the chromosomes becomes affected at an earlier period, that of the figs. 17 and 18. There is evidence that in *Syrbula*, as I have shown to be the case in *Peripatus*, there is a continuous linin spirem during the synapsis stage; but at no period of the first spermatocyte is there a continuous chromatin spirem. The splitting of the chromatin globules does not occur simultaneously for all composing a chromosome, but rather successively; and each globule or granule is a mass of demonstrable smaller microsomes. Hence there is no proof that each smallest visible microsome divides into two during the longitudinal splitting of a chromosome.

Then comes a post-synapsis stage in which the chromosomes are no longer densely grouped, and when the longitudinal split is very clear. In figs. 23 and 24, illustrating this stage, only three and four respectively of the nine bivalent chromosomes are drawn; and the point x on each marks the linin band connecting every two univalent chromosomes. Very rarely does the longitudinal split become wider than shown in these figures, but sometimes it widens as much as is shown in the largest chromosome of fig. 25. This was the maximum extent of separation seen of the halves of a split univalent chromosome, and from this stage through the following this split narrows gradually.

Unlike most of the Hemiptera no rest stage follows, but the spermatocytes enter immediately upon the prophases of the first maturation mitosis; successive steps of this process are shown in figs. 27–31. The nucleus enlarges, the chromosomes lie close to its wall, the delicate linin fibres change their character and break each into a row of minute

globules, as roughly indicated in fig. 29; this last phenomenon I have found to be of general occurrence in spermatocytes, but it has been investigated most in ovocytes. The chromosomes through these stages shorten and condense, some into bent or straight rods, others into more or less closed rings; the surface of the chromosomes remains rough and somewhat filamentous until the nuclear membrane disappears (compare fig. 30 with fig. 31). The longitudinal split of the chromosomes gradually narrows, as one sees in the series of figures 23–25 and 27–30; it does not widen out, so that the relations of the univalent components of a bivalent chromosome remain approximately the same as in preceding stages. The early bivalent U or V of the synapsis period may become a straight dumbbell, or its univalent arms may become apposed along their length, or it may become a ring; but in all cases the position of the longitudinal split is along the length of each univalent chromosome, whether that be straight or bent (figs. 27, 28, 30). Very rarely have the chromosomes an X-shape (fig. 31). Therefore each bivalent chromosome is composed of two univalent chromosomes joined by one end or by both ends (in the case of rings), and the space enclosed by a ring is not a longitudinal split but the area separating two entire univalent chromosomes. Where the two univalent chromosomes of a pair are connected is in most cases marked by a constriction (*x*, figs. 27, 29), and in the rings there may be two such constrictions (the larger ring of fig. 29) in accordance with the conjunction in these cases of both ends. These chromosomes are thus essentially, in formation and shape, like those of the Hemiptera and *Peripatus:* each represents two longitudinally split univalent chromosomes joined by one or both ends. And the gradual narrowing or closure of the longitudinal split is as evident and undeniable as in any other object studied by me.

Through these prophases the heterochromosome is recognizable by its smooth contour and compact structure (Plate IX, *n. 2*, figs. 27–30). It is now almost always in the form of a rod so bent that both arms lie contiguous and parallel, as shown in fig. 26 where the arm seen on high focus is stippled and that seen on deeper focus drawn in outline only. Each of its arms, as we have seen, represents a univalent heterochromosome.

With the disappearance of the nuclear membrane, which commences to dissolve away first at the poles near the centrosomes (Plate IX, fig. 31), the chromosomes have attained their completed dense structure and smooth outline and take their position within the equatorial plate (figs. 32–34). There are exactly ten bivalent chromosomes present, one-

half the number of univalent chromosomes present in the spermatogonia, namely, one bivalent heterochromosome and nine bivalent ordinary chromosomes. But at this stage there seems not to be possible a positive recognition of which is the heterochromosome. In a number of cases after nine of the chromosomes were arranged in the equator and some of them were beginning to divide (fig. 33), one (*y*) had not yet taken up that position but lay nearer one spindle pole than the other. This was the case, *e.g.*, with four cells in exactly the same stage lying in the same section of one testicular follicle, and in all of these the isolated chromosome was of the same size and form, straight, and appearing to consist of two closely apposed arms. It may be that this chromosome is the heterochromosome with which it agrees in general form and size, but this could not be definitely determined; ultimately it takes a position in the equator and divides with the others. In fig. 32 is an element, *y*, closely corresponding in size with it and with the heterochromosome during the prophases; but I cannot say positively that *y* of fig. 32 is the heterochromosome, though the probability of it is evident. All these chromosomes become so placed in the spindle that mantle fibres from one spindle pole are attached to one univalent element, and mantle fibres from the other spindle pole to the other univalent component of each bivalent chromosome. The longitudinal split can no longer be seen, but previously it lay in the axis of each univalent chromosome. These definitive chromosomes may be dumb-bell-shaped, or as frequently irregularly V-shaped, ring-shaped, or in the form of two parallel rods (Plate IX, figs. 31–33; Plate X, fig. 34). But whatever the form, they become arranged so in the spindle that the point or points of junction of the univalent components of each lies in the equatorial plane. The early formation of these chromosomes, their arrangement in the spindle, then their division (Plate X, figs. 34–36) show that the first maturation mitosis is a reduction division and separates from each other the univalent chromosomes of each pair. There is no evidence that this is an equation division taking place along the line of the longitudinal split—no evidence at any period that a chromosome had become elongated in a line at right angles to its original long axis. Each arm of a bivalent chromosome is a whole univalent chromosome and not a split half of one; and the long axis of each arm is in the same line as its long axis at earlier stages. I have tested the morphological evidence of this process very honestly and fairly, for at the commencement of my study I was quite prepared to find the first maturation mitosis an equation division. But it is a reduction division. The division of certain of the chromosomes may

call for some further explanation. Next to the straight or slightly bent dumbbell-shaped bivalent chromosomes the most frequent form is that of an irregular V, such as those lettered *K* in Plate IX, figs. 32 and 33; in each of these figures one univalent half of each such chromosome is shown black and the other white; *p* of fig. 32 is such a chromosome seen at right angles to the other views. *K* of Plate X, fig. 34 shows the separation of the components of such a chromosome. The division of one of the forms of *y* of Plate IX, fig. 32 is shown by *y* in Plate X, fig. 34. The division of the dumbbell-shaped chromosomes is clear from the figures. Whereas ring-shaped chromosomes are frequent in the preceding late prophases, they are only very exceptionally found in the equatorial plate, so that probably by the pull of the mantle fibres upon them these rings change into the form of the chromosomes lettered *K*.

In the anaphase of this reduction division as homologous univalent chromosomes move apart from each other, each opens up in the form of a V (Plate X, figs. 35–37). This opening is the reappearance of the longitudinal split, since it is a cleft along the long axis of each univalent chromosome. In no way can it be considered a transverse split, a space between two whole univalent chromosomes. This split is widest and appears first at the end of the chromosome turned toward the equatorial plane, and rarely extends quite through the opposite end. Vertical (fig. 38) and obliquely lateral (fig. 37) views of a daughter plate of chromosomes, *i.e.*, of the chromosome plate of a second spermatocyte, show without exception ten elements, the same as the number in the first spermatocytes; accordingly all the chromosomes divide in the reduction mitosis. But each of the ten elements of the second spermatocyte is univalent instead of bivalent, and its cleft or constriction marks the longitudinal split. Without any indication of a rest stage the centrosomes of each second spermatocyte wander apart from each other, and each through an angle of 90°, so that the axis of the second maturation spindle comes to lie at right angles to that of the first (fig. 39). In the equator of this spindle each of the ten chromosomes becomes so placed that the line of its longitudinal split coincides with the equatorial plane. In the ensuing anaphase occurs, then, an equatorial division, separation from each other of longitudinal halves of univalent chromosomes. All ten chromosomes divide, and a pole view of one of the resulting daughter cells (spermatids) shows also ten chromosomes (fig. 40), exactly half the number found in the spermatogonium; no exceptions were observed to this numerical relation.

Exactly how the bivalent heterochromosome comports itself in the

maturation mitoses could not be definitely settled, since it could not be satisfactorily distinguished from other chromosomes of about the same size. But there is some probability, as was pointed out, that it may be the chromosome marked *y* in Plate IX, figs. 32 and 33; and in very early stages of the spermatocytes (figs. 15, 16) it showed a longitudinal splitting which soon after seemed to disappear. For these reasons of its proved bivalence and its longitudinal splitting, in conjunction with the fact that each chromosome divides in each maturation mitosis, it becomes most probable that it undergoes a reduction division in the first mitosis, and an equation division in the second. For since it is formed and has essentially the same constitution as the other chromosomes, there would be all reason to expect it to divide like them; and a more trenchant reason is this, that of the ten chromosomes of a second spermatocyte there is no particular one which from any peculiarity of structure could be regarded as bivalent. This is, of course, only circumstantial evidence of its undergoing first a reductional and then an equation division, but the probability of this contention is obvious; there is no doubt that it undergoes two divisions.

In the monaster stage of the spermatogonia (Plate IX, figs. 7, 8) certain chromosome pairs could be recognized by peculiarities in form and size, namely, those lettered in these figures. It is corroborative evidence of the persistence of the individuality of the chromosomes, if, indeed, any further proof of this idea is needed to-day, that the same differences are observable in later stages. So among the ten univalent chromosomes of a second spermatocyte (Plate X, figs. 37, 38) are found three notably larger than the rest and three markedly smaller. So in the figures one marked *F* (*f*) would correspond either to *F* or *f* in Plate IX, figs. 7, 8; *A* (*a*) to either *A* or *a* of figs. 7 and 8; and so on for the others. And even in the spermatid (Plate X, fig. 40) there are the same size relations; the ten pairs of chromosomes of a spermatogonium could be obtained by putting together the ten chromosomes from each of two second spermatocytes derived from the same first spermatocyte; but the ten single chromosomes of a second spermatocyte could be reëstablished only by bringing together the ten semivalent chromosomes from each of the two spermatids resulting from such a spermatocyte. The first maturation mitosis separates from each other the two univalent chromosomes that compose a pair of dissociated ones in the spermatogonium, and a conjugated pair in the first spermatocyte.

Finally, a word as to the behavior of certain cellular structures other than chromosomes—only a brief statement, for the present results are

corroborative of my (1900) earlier ones upon *Peripatus*. In the achromatic spindle a central spindle, fibres continued from pole to pole, but not attached to chromosomes, is found in the spermatogonia, but not in the spermatocytic mitoses; its fibres and the pole fibres are formed from the cytoplasm. The mantle fibres, on the other hand, those connecting the chromosomes with the centrosomes, are derived from linin fibres previously connected with the chromosomes—the mantle fibres are at least in great part nuclear in origin. Whatever be our views upon the nature of the pole and central fibres, whether we regard them as lines of currents or as actual fibrils, I think we must consider the mantle fibres as contractile fibrils, not simply paths of movements of fluids. This follows clearly from the results of my *Peripatus* paper, where the mantle fibres of mitosis were shown to be derivable from linin fibrils stretched out through the nucleus in the rest stage, and there constituting a continuous linin thread (spirem) with many fine collateral branches. Such fibrils crossing one another in all directions in the resting nucleus cannot be considered current-paths; how, then, change into current-paths during mitosis, except in so far as we regard a contracting gum-elastic cord to be a path of movement? What holds for the mantle fibres need not, however, obtain for the pole and central spindle fibres. The other achromatic spindle constituents of nuclear origin are the connective fibres, fibres pulled out between two separating daughter chromosomes; these are clearly derived from the linin forming the matrix within which the chromatin of a chromosome is imbedded or the sheath by which it is surrounded. When two daughter chromosomes separate in metakinesis it has the appearance as though two connective fibres pass between them; but it is more likely that such two lines represent in actuality the boundaries (visible because of their higher refraction) of a solid or hollow linin cylinder.

Already in the monaster stage of the spermatogonia (Plate IX, fig. 9) as of the first spermatocytes (fig. 32) there is a pair of centrosomes at each spindle pole. After each spermatogonic division these centrosomes wander through an arc of 180° to take up a position on the opposite side of the nucleus; the same process seems to take place in the spermatid; but before the second maturation mitosis each wanders through an arc of only 90°. In the first maturation mitosis there are two mantle fibres from each spindle pole to each chromosome (Plate IX, figs. 32–33; Plate X, figs. 34–36); in the second only one (fig. 39). This is understandable on the basis that the first mitosis is reductional, since, as is most clearly shown in a straight dumbbell-shaped bivalent

chromosome, one univalent chromosome is turned toward one spindle pole and the other toward the opposite one, and each univalent chromosome being longitudinally split the linin thread attaching it to the spindle pole must be split into two; for any longitudinal splitting of the chromatin globules is always associated with, if indeed not induced by, a splitting of the linin matrix, as is shown in the details of chromosome formation, especially in the post-synapsis stage. If the first maturation division were equational there would be no adequate explanation for the double mantle fibres. As the centrosomes of the second spermatocyte move apart preparatory to the second maturation mitosis, each carries along with it one of the two mantle fibres attached to each chromosome.

2.—Spermatogenesis of Lycosa insopita Montg.

The only published work upon Aranceæ is that of Carnoy (1885), Wagner (1896) and Wallace (1900). Miss Wallace's paper was done partly under my direction, and is a short preliminary note dealing with the accessory chromosomes, which she correctly found to be double in the spermatocytes, but did not determine positively its behavior in the maturation mitoses. Wagner's short paper I have not at hand, and cannot now recall his conclusions. Carnoy described quite minutely the process of cell division in male germ cells of a number of spiders, of *Phalangium* and *Scorpio*, illustrating both spermatogonic and spermatocytic divisions (his whole plate V with its numerous beautiful figures); such work has no direct bearing upon modern spermatogenetic study, in that it does not consider the sequence of changes through the several cell generations. But it is but just to say of this study of this priest of Louvaine, that it was in many respects the best work of its day upon cell division. Most writers, following a certain antiquated German school, have neglected to refer to him. But he pointed out that cell division is not all of one kind, but that two main types of it occur, and this we now know to be the case and term them respectively *reduction* and *equation* divisions; yet this was the main ground on which that particular German cult fought him. And he undoubtedly saw much more than many a later investigator, and lacked only a general standpoint of interpretation.

Testes were studied of an adult male caught in October; they were fixed and stained by the methods used for *Syrbula*. The testes are slender, cylindrical tubes, and on account of the difficulty of removing them entire from the fresh animal, one proceeds best by cutting off the abdomen from the living animal, removing the hypodermis, fixing the

whole abdominal visceral mass, then dissecting out the testes in distilled water.

The spermatogonia form the inner epithelium of the organ, and by their last divisions the spermatocytes formed lose their connection with this cell layer and come to lie free within the lumen of the testis; in this cavity are found all stages of the growth period, the maturation mitoses, the spermatids and spermatozoa. In any transverse plane of a testis one finds the same series of stages. Not only in the arrangement of the cells within the testis, but also in the process of spermatogenesis this spider shows close resemblance to *Peripatus;* in both, *e.g.*, the longitudinal split of the bivalent chromosomes is very clear, and during the synapsis stage the chromatin loops are not so densely massed but that each may be distinguished.

Only two clear cases of pole views of the equatorial plate of spermatogonia were found. On one of these (Plate X, fig. 41) exactly twenty-eight chromatin elements could be distinctly counted. In the other case the chromosomes were more densely grouped, and I could not be certain whether there were twenty-eight or thirty of them; it was possible that two of them were already dividing in metakinesis. Two of these spermatogonic chromosomes are very small (*S.*); the subsequent history of these could not be ascertained with any degree of certainty. There are accordingly twenty-six larger chromosomes, all of which can be recognized in the thirteen bivalent chromosomes of the first spermatocyte. All of these appear to be longitudinally halved during the anaphase, so that each first spermatocyte receives twenty-six daughter chromosomes.

There is no rest stage at any period of the spermatocytic history. Shortly after the last spermatogonic mitosis commences the synapsis stage (figs. 42–44). At its beginning (fig. 42) the daughter chromosomes are elongated threads, already commencing to join into pairs (at the points lettered *x*). But two of them (*N. 2*) differ in maintaining the dense contour and smooth outline characteristic of mitosis; these are the heterochromosomes, and there is clearly one pair of them. Accordingly, of the twenty-six large chromosomes of the spermatogonia two are heterochromosomes, though they cannot be recognized in the spermatogonic monaster stage nor yet in the preceding rest stage. In following synaptic stages (figs. 43, 44) the twenty-six chromosomes unite to form thirteen bivalent pairs. This takes place, as in *Peripatus,* by an approximation or even close fusion of every two chromosomes of similar length at their ends directed toward the central pole of the nucleus (that one farthest removed from the greatest mass of cyto-

plasm). During this process there appears to be a continuous linin spirem, but no continuous chromatin spirem; the bivalent chromosomes in the form of V's or U's are arranged upon the linin thread so that their free ends are toward the distal nuclear pole. The distal ends of each V are rarely contiguous with those of a neighboring one, though that is sometimes the case, but, as the figures show, they are usually slightly separated. Between the distal end of one univalent component of a V and the corresponding end of a similar component of another V can be seen, whenever these structures lie in the same plane, a connecting linin thread. These phenomena are so similar to those in *Peripatus* that I think it unnecessary to describe them all in detail again, and refer to the very detailed account of the *Peripatus* paper. A pole view of a nucleus in the synapsis stage shows the optical cross-section of twenty-six chromatin loops, and lateral views demonstrate the presence of thirteen V's. Each V, therefore, corresponds to two univalent chromosomes of the spermatogonia; it is a bivalent structure in which each arm represents one chromosome, and has been formed by the conjugation of two end to end and not by transverse scission of a continuous chromatin spirem. Where the ends of two conjugated univalent chromosomes come together (the points marked x in these and the subsequent figures) is frequently found a slight notch or break, which is a connecting band of linin—corresponding to the *central* linin band in *Peripatus*. That the space between the two arms of a V is not a longitudinal split is indubitable, for stages like that of fig. 42 show previously separated chromosomes coming together. The longitudinal split appears in the long axis of each univalent chromosome (figs. 42–44), and proceeds latest to their distal ends (those directed toward the distal pole of the nucleus). No earlier longitudinal split occurs, and no later one. A nuclear membrane appears first at the close of the synapsis.

The behavior of the heterochromosomes can be followed with equal facility and certainty. All through the growth period they preserve their smooth contours, compact structure and strong affinity for chromatin stains. The two univalent heterochromosomes (*N. 2*, fig. 42) come together and conjugate side to side (figs. 43, 44), though their ends directed toward the distal nuclear pole are in closer touch than their opposite ends, in contrast to the behavior of the other chromosomes. So is produced a bivalent heterochromosome, throughout the growth period placed against the distal pole of the nucleus, consisting of two univalent chromosomes lying parallel or more usually in the form of a much narrowed V. It does not increase in size in the following stages,

and its only perceptible change is a longitudinal split of each univalent component. This is shown in fig. 44*bis*, *A*. and *B*, which show merely the bivalent heterochromosome and an arc of the contiguous nuclear membrane. In most cases the space of this split is widest at the inner ends of the univalent components of this bivalent heterochromosome, as shown in fig. A. This longitudinal split cannot be seen from every point of vision, but only when the heterochromosome lies in particular directions, as is quite understandable.

Following the synapsis is a post-synapsis stage, with the bivalent chromosomes more evenly distributed through the nucleus (figs. 45, 46). The longitudinal split is wide and very evident, but does not extend through the distal ends of the still generally V-shaped loops. In each bivalent chromosome the angle of the V (*x* of the figures) is the point of junction of two univalent parts. Here also the longitudinal split of the bivalent heterochromosome (*N. 2*) can sometimes be seen. No nucleolus is formed in any part of the growth period.

Immediately succeeding are the prophases of the maturation mitoses (figs. 47–52). There occurs in them a gradual shortening and condensation of the chromosomes, leading to narrowing or even complete temporary obliteration of the longitudinal split. This split in the early prophases (figs. 47–49) in the case of some of the chromosomes becomes a little wider than during the post-synapsis (figs. 45, 46); so with the chromosomes marked *H* in these figures. But this happens with only a minority of the chromosomes in any nucleus. And it is not a definite stage in the structural change of every chromosome, for the reason of its relative infrequency. Most of the chromosomes, on the contrary, are straight or bent rods, and the angle or middle point of these marks the point of conjunction of two univalent chromosomes (*x* of the figs. 47–52). Such chromosomes as those marked *H* in figs. 47 and 49 are ones where the longitudinal split has become very wide at the point of union of the two univalent chromosomes; but even in such chromosomes one axis always remains longer than the other, so that there is no evidence of a bivalent chromosome becoming extended out in a line at right angles to its previous long axis. And even for these chromosomes, as is clearly the case with the others where there is no extensive widening of the longitudinal split, the successive prophases lead toward a narrowing or closure of this split. Regular rings appear not to be formed. But chromosomes in the form of an X are not infrequent. There is no difficulty in the interpretation of the form of these. For in the one shown in fig. 51, marked *D.*, the X is seen to be two univalent chromosomes, each longitudinally split, joined by their middle points;

an X is then obviously formed from a V, not by any extension of the longitudinal split, but simply by the point of contact of the two univalent chromosomes shifting its position. The decisive phenomenon through these prophases is that most of the chromosomes preserve their original forms of bent rods, or modify them into straight rods, leading toward the forms most frequent at the end of the prophase (fig. 52), where the earlier point of union of two univalent chromosomes is recognizable (x), and the longitudinal split sometimes still discernible. For the greater number of the chromosomes the changes of the prophases lead to the retention of approximately their original form, but with a gradual partial or complete closure of the longitudinal split; and there is no reason to hold that the longitudinal split ever widens and remains open in such a manner as to change the position of the long axis of a bivalent chromosome. The heterochromosome undergoes no marked modification during the prophases; at first each of its univalent portions shows still the longitudinal split, shown in end view on fig. 49 ($N.$ 2), but toward the close of this period this split appears to close up.

In the equatorial plate of the first spermatocyte (figs. 53, 54) are found thirteen larger bivalent chromosomes, and sometimes a minute chromatin body ($S.$) which does not appear to be bivalent, at least it is not bipartite. The latter may represent one of the two minute chromosomes of the spermatogonia (fig. 41); one of these small bodies is occasionally found in the monaster stage of the second spermatocytes (figs. 62, 63). Their behavior in the growth period could not be determined, so we must disregard them in our analysis of the chromosomal relations. The first maturation figure has then thirteen bivalent chromosomes, corresponding to the twenty-six larger univalent ones of the spermatogonia. Lateral views of the first maturation spindle are shown in figs. 55–58. While on most of the chromosomes at this stage the longitudinal split is not evident, in some cases it is still persistent, as notably in those lettered K in figs. 55, 56, 58. Particularly the one in the last figure is valuable in demonstrating how the chromosomes become placed in the spindle: the point of junction (x) of the two univalent components lies in the equator, therefore the one univalent chromosome just above and the other just below this plane. From this arrangement and from the mode of insertion of the mantle fibres it is evident that in this mitosis the two univalent chromosomes of each pair become separated from each other into opposite cells, and that this is a reduction division. Generally the long axis of each bivalent chromosome is parallel to the axis of the spindle, which is always

the case when its univalent parts are placed in one line. Sometimes, as with the extreme right and left ones of fig. 57, the long axis of the chromosome appears to lie in the equatorial plane; this results also in a reduction division, however, because here there is a bent instead of a straight bivalent chromosome, with consequent convergent disposition of the two univalent chromosomes. *Lycosa* is particularly demonstrative of this first mitosis being a reduction mitosis, on account of the simple form of the chromosomes and of the occasional perceptible persistence of the longitudinal split at this stage. No chromosomal rings occur at this stage; the nearest approach to them are oval forms like the two largest in fig. 55, with very exceptional width of the longitudinal split; such forms are individual variations, not found in every cell, as one sees on comparison with pole views (figs. 53, 54) showing all the chromosomes, yet even in them the original long axis of the chromosome is recognizable.

In metakinesis (fig. 59) all the bivalent chromosomes undergo a reductional halving. Figs. 60–63 show pole views of the chromosomal plates of the daughter cells, second spermatocytes. Disregarding the two minute bodies (*S.*) of 62 and 63, we find in 60 fifteen chromosomes, in 63 fourteen, in 61 thirteen, in 62 twelve. There would then seem to be a range in number from twelve to fifteen. This I believe is due rather to some unexplained individual variation than to the possibliity of a normal unequal distribution of the chromosomes. For in the nine cases where they could be easily counted the numbers fifteen, fourteen and twelve were each represented by only one case, whereas thirteen appeared in six cases; and in the only two cases where the chromosomes of the second spermatocyte could be counted on lateral view (one of these shown in fig. 65) there were in both cases thirteen chromosomes. There were thirteen bivalent chromosomes in the first spermatocyte, and the counts show that in the majority of cases, so probably as the normal phenomenon, there are thirteen univalent ones in the second spermatocyte. As the chromosomes of the first spermatocyte separate in the anaphase (fig. 59) each daughter chromosome shows a divergent split widest at the equatorial end; this can be nothing else than the reopening of the original longitudinal split, if one compares the appearances in fig. 59 with the chromosome most to the left in fig. 55. So each bent chromosome of the second spermatocytes (figs. 60–65) is a univalent chromosome so split longitudinally that the cleft is narrow at one end and widens out toward the other. There is no proof of any kind that this is either a transverse break or a line of separation between whole univalent chromosomes.

The second maturation mitosis (figs. 64, 65) is accordingly an equational division. I have been unable to count the chromosomes in the resultant daughter cells, spermatids, because of their massed arrangement there, and therefore have not demonstrated that all the elements become halved in this second mitosis. But all these thirteen chromosomes are constricted or cleft, showing that each is therefore probably longitudinally split; for this reason it is probable that each spermatid receives thirteen chromosomes.

As was the case in *Syrbula* so also in *Lycosa* the mode of division of the bivalent heterochromosomes was not positively determined. In the spermatocytes it can always be distinguished by its smooth outline and compact structure only up to the time when the other chromosomes acquire their final shape. Yet among the chromosomes of the first mitosis there seems to be no particular one markedly different from the others. But on lateral view of the spindle (fig. 56) there is sometimes one (*t*) quite different from the others, in the form of two elongated rods; and its division is shown in fig. 58 (*t*). This may be the heterochromosome, but there is no satisfactory evidence for this conjecture. We found that in the synapsis this was formed, like the ordinary chromosome, by a conjugation of two univalent ones, and that each univalent one underwent a longitudinal splitting. This similarity in formation is some evidence that the heterochromosome may behave like the others during the maturation mitoses, namely, that it may undergo a reductional division in the first and an equational in the second mitosis. And we can say positively that the whole bivalent heterochromosome does not pass undivided into one of the second spermatocytes.

In both maturation divisions the centrosomes of both spindle poles touch the cell membrane (figs. 55–59, 64). As in the other objects studied by me there is no intermediate cell plate formed after the reduction division, but after all other divisions.

3.—Occurrence and Time of the Reduction Mitosis.

Korschelt (1903), in his excellent review upon the maturation phenomena, distinguishes two types of maturation: the "eumitotic," where both mitoses are equational, and the "pseudomitotic," where one of them is reductional. But these should be considered collective terms for groups of divergent opinions, rather than a classification of actually occurring natural phenomena. The general consensus of opinion at the present time, the greater part of all the more recent work on most diverse animals, is conclusive for the decision that a reduc-

tional mitosis, a separation of whole univalent chromosomes, occurs in many objects. In all the works where two successive equational divisions have been described, it is significant that no positive explanation has been given of the earliest mode of origin of the bivalent chromosomes, not even in the detailed study of Brauer (1893), and the same may be said of the recent elaborate analysis of de Sinéty (1901). All the "eumitotic" investigators seem to have interpreted as a first longitudinal splitting of the chromosomes a space which they have not proved to be such, and which the observations of others show to be in all probability the space between two conjugated univalent chromosomes. They likewise fail to account for the fact that the chromosomes in the reduction divisions so frequently differ in form from all other chromosomes, and leave undecided the question of the origin of the bivalent chromosomes. It is not necessary to go further into detail here upon this point, on which I have expressed myself many times previously. But we can say positively that there has not yet been proved any case of eumitotic maturation in the sense of Korschelt: that even in *Ascaris*, the foundation-stone of this doctrine, Sabaschnikoff (1897) has shown that Boveri (1888), Hertwig (1890) and Brauer (1893) may have given a wrong analysis, while recently Boveri (1904) himself and I (1904*a*) have argued for the probable occurrence of a reduction division here; and for the vertebrates also King (1901), Schreiner (1904), Maréchal (1904) and I (1903, 1904*a*) have proved the same contention. While this dispute will not be settled for some time, for the reason that scarcely a beginning has yet been made in the study of the germ cells, I do not hesitate to declare that in none of the Metazoa does maturation of the eumitotic type occur. And I make this prophecy after starting from the point of view (1898) that there may well be different modes of maturation, and consequently I can surely not be accused of starting out on my studies with bias in any particular direction.

Further, all evidence of any strength is to the effect that probably in no case are both maturation divisions reductional. This standpoint has been held by Wilcox (1895) and a few others, and by myself in my first paper (1898), but I quickly discovered and corrected this initial error (1899). To this "Correction" another correction must be made: in the note of 1899 I wrote that the second maturation may be occasionally reductional, occasionally equational; this was a mistake, for now I can say there is in *Pentatoma* (*Euchistus*) no evidence at all of reductional division in the second mitosis.

All maturation modes are then of the pseudomitotic type, and of

these Korschelt distinguishes a "Prereduction," where the first mitosis is the reductional one, from a "Postreduction," where the second one is reductional. Until quite recently I held that it was not of great physiological importance which of these mitoses was reductional, provided that in all cases one of them was. But my own studies, extended over a diversity of objects, have convinced me that very probably there maintains a uniformity here also, even though the understanding of it may not be immediately forthcoming. For surely out of the endless diversity in small details a larger uniformity is gradually showing itself, and as scientific thinkers it is our object to discover the uniformity. The minutiæ of phenomena are but stepping-stones, and too often slippery ones, toward simple and broad concepts. By analogy with other natural phenomena we should *à priori* expect uniformity rather than diversity. From this standpoint I enter upon the discussion again, with the conviction that all maturation plans must be either prereductional or all postreductional, and that there can be no compromise. In our decision we must argue from the facts presented in the early growth period of the ovocytes and spermatocytes, from their first mode of formation and axial relations to each other, and not from analyses of their definitive forms. Other things being equal, such evidence must have the most weight which considers in the greatest detail the full sequence of stages, and most particularly such as treats minutely the early growth period. Too often follows upon a short and incomplete series of observations a long discussion in print of possibilities and probabilities, like shuffling with an incomplete pack of cards, instead of an attempt to settle the matter with the microscope.

The foundation of the argument for postreduction is in the work of Häcker and Rückert upon the Copepoda, and with but few exceptions this has been allowed to go unchallenged. On that account the facts of these workers call for careful examination.

Rückert (1894) studied the ovogenesis in three genera from the stage of the young ovocyte up to the monaster stage of the first maturation mitosis. From the number of chromosomes present in young blastomeres of one of these, he concluded that each chromosome of the ovocyte is bivalent, equal to two. He described most fully *Cyclops strenuus*, so that we will examine his work upon this species. His figs. 4–8 present stages of the earlier growth period, by no means a complete series. The first ovocyte in these stages has eleven chromosomes, each in the form of two more or less closely apposed rods. Rückert assumes that each of these is a longitudinally split chromosome; but while he correctly assumes that each is bivalent, he does not determine the

boundaries of each univalent component at this stage, but from conditions in later stages (his figs. 9–22) concludes that the middle point of each double rod is such a boundary. This is the weak point in his whole analysis. For why may not each of the single rods be a whole univalent chromosome, the bivalent one being formed then by a conjugation side to side of two univalent ones? None of his figures in the least exclude this possibility. And here may be recalled Lerat's (1902) somewhat inclusive observations also upon *Cyclops,* to the effect that each such double rod may be so constituted. Then there is a great break in Rückert's observations between the stages of his figs. 8 and 9, the one apparently a post-synapsis or equivalent early stage, the latter an advanced prophase; yet within just this undescribed period we would expect great changes in the form of the chromosomes, such, for instance, as the appearance of an indubitable longitudinal split. He states that each double rod in the prophase bends at the middle, and later breaks transversely into two at this point; this he conceives to be a separation of the univalent chromosomes at the point at which they had hitherto been united. But he presents no positive evidence, certainly not in his drawings, that this is not the appearance of a longitudinal split of each univalent element, a split whereby the halves would remain attached at one end and gradually separate at the other (just as has been described for other objects), opening up from a narrow V to a condition in which the separated halves of one univalent chromosome come to lie together in one straight line. The uppermost chromosome of his fig. 11*b* is evidence of such a possibility. Accordingly, though the bivalent chromosomes lie so in the equator of the first pole spindle that their long axes coincide with this plane, and their "transverse" splits are at right angles to it, this does not prove the first mitosis to be equational. For all the proof he brings to the contrary, the opening along the length of each bivalent chromosome may be a line separating its two univalent components, and the first division therefore reductional. Rückert does not convince, though his is in many respects the most careful work yet done upon these forms, because of the hiatus in his stages, and because, and this is the cardinal issue, he failed to decide the mode of origin of the bivalent chromosomes.

I have not at hand Häcker's earlier papers (1891, 1892, 1893), and so quote from Rückert (*l.c.*) his opinions: "Zuerst hat er die Reduktion in die erste, dann in beide und zuletzt in die zweite Teilung verlegt." In later papers (1895, 1899) he confirms Rückert's view that the reduction is effected in the second maturation mitosis; though I cannot see that in so doing he brings any stronger proof than did

Rückert. Change of view is no dishonor but a sign of courage, and I respect any man for it, for it is not easy to discard an idea for which one has fought; but in scientific thought we expect change of view to be an accompaniment only of the discovery of more urgent facts. Such facts we do not find in Häcker's latest work (1902, 1904). He had previously observed that in *Cyclops brevicornis* the normal number of chromosomes is twelve, and that in the ovocytes before the maturation divisions there are six bivalent ones; that these divide equationally in the first and reductionally in the second spindle, so that the ovotid receives six univalent ones. But now he maintains this is wrong, that the chromosomes at all periods of the first ovocyte while bivalent are in the normal number, and that there is no union of them into pairs during the growth period. I have already criticised (1904*b*) this view, and in his retort (1904*b*) Häcker has failed to take up the cardinal issue and give further proof. He describes (1902) that in the ovocyte these twelve bivalent chromosomes are arranged in two planes of six each. This is not borne out by his figs. 30–34. He came to this strange conclusion in a roundabout way from observations upon the gonomerity of the nucleus, holding that even at the time of the first maturation division the chromosomes are arranged in two planes, corresponding to the earlier gonomeres of the nucleus, one layer of them being maternal and the other paternal. The only evidence for this are certain lines or septa said to divide the "provisory division figure" transversely and longitudinally. No one has corroborated the existence of such septa, and I have looked in vain for them upon a number of objects; he gives only lateral views of these structures, does not show their origin, and does not make it plain whence their substance is derived. Yet, fairly speaking, this may be said to be the whole observational basis for his new involved analysis! Each ovocyte of the second order is said to receive twelve bivalent chromosomes; and then follows a union of tetrads into pairs. "Bei der ersten Richtungsteilung gelangen, wie bei jeder anderen Kernteilung, je 6 väterliche und 6 mütterliche Elemente in die Tochterkerne, jedoch erfolgt die dicentrische Wanderung nicht in zwei gesonderten, den elterlichen Anteilen entsprechenden Gruppen, sondern die väterlichen und mütterlichen Elemente müssen, ihrer Aufstellung in den zwei Fronten entsprechend, zwischen einander durchtreten und sind also vollkommen durcheinander gemischt, während sie an die Pole wandern (Textfig. *Cb*). Diese Mischung ist jedoch, wie wir gesehen haben, keine unregelmässige. Denn es liesst sich mit grösster Wahrscheinlichkeit zeigen, dass bei der unmittelbar folgenden Paarung der Spalthälften die Paarlinge jeweils zwei im

sekundären Keimbläschen einander opponierten Vierergruppen angehören. Es muss sich also schon die dicentrische Wanderung in einer ganz gesetzmässigen, Quadrillenähnlichen Ordnung vollziehen, mögen dabei regulierende, von den Chromatinelementen selbst ausgehende Reize oder irgend welche als Leitbahnen dienenden Kernstrukturen eine Rolle spielen. Bei der Paarung der Spalthälften erfolgt die Vereinigung je einer väterlichen und einer mütterlichen Spalthälfte. Von den beiden einander opponierten Vierergruppen $\frac{ab}{ab}$ und $\frac{no}{no}$ werden sich z. B. jeweils zwei Spalthälften ab und no miteinander verbinden und das Gesamtresultat des sersten Teilungschrittes ist demnach eine gleichmässige Durchmischung der väterlichen und mütterlichen Anteile (Textfig. *Cb* unten)." This recalls Fol's (1891) quadrille of the centrosomes! The result of it all amounts to this: spermatid and ovotid have each six bivalent chromosomes, the fertilized egg has twelve bivalent chromosomes, and the same number is found in the first polar spindle where they are arranged in two planes; each second ovocyte receives twelve bivalent chromosomes, and there unite into six quadrivalent chromosomes; and these undergo a reduction division in the second mitosis, so that the ovocyte receives six that are bivalent. This analysis is so intricate and complex, so little borne out by the fragmentary and somewhat doubtful evidence—only certain lines traversing a nucleus—that we can charitably say the paper is its own strongest critic. It is to be much regretted that Häcker has used these results in a general review (1904) of bastardization, because they are irreconcilable with all other work, and tend to make the supposed diversity and contradictoriness of the germ cell phenomena even more marked than ever before. We are not in any need of "Referate," but very pressing need of more observations.

The work of Linville (1900) on *Limnæa* is not conclusive, for the chromosomes are very minute and the prophases were not studied at all; the same may be said of Francotte's (1898) study of Polyclades, where the only figures are indistinct microphotographs. The investigations of Van der Stricht (1898) and von Klinckowström (1897) upon Polyclades have been strongly contradicted by Schockaert (1902), who has given a much more detailed examination than either of these writers. The papers of Prowazek (1901, 1902) I have not seen. Miss Wallace's paper (1900) is admittedly indecisive, and Griffin's (1899) studies on *Thalassema* and *Zirphæa* concern chromosomes of very intricate forms and small size, and their behavior was elucidated (or should we say nigrified?) by an analysis of their final shapes. So none of these investigations are decisive in any manner that requires rigid proof from a study of the whole series of changes.

There remain certain studies upon the spermatogenesis of insects, the most deserving of attention of which are those of McClung and Gross. Vom Rath's studies of *Gryllotalpa* (1892, 1895) omit all the earliest stages of the growth period; and while he takes the stand that the maturation is postreductional, he grants the possibility of its being prereductional. McClung (1900, 1902) holds the postreductional viewpoint, reasoning particularly from the forms of the definitive chromosomes; in the late prophases of the first spermatocyte the bivalent chromosomes vary much in shape, rods, rings, crosses, and apparently intermediate conditions. There is more uniformity in the first maturation spindle. These differences in form McClung interprets as successive stages and, to put it concisely, he argues that the axial relations of a chromosome change, so that if the long axis were originally from right to left, it subsequently changes into a line at right angles to this. X-shaped chromosomes are thereby interpreted as intermediate stages in this transformation. His figures of Acridid spermatocytes are very similar to those I give in the present paper of *Syrbula* of the same family of the Orthoptera; but McClung holds that an elongate bivalent chromosome placed with its long axis parallel to the first mitotic spindle undergoes an equational division, therefore that the line of separation of its univalent components lies along its length. From the assumption that the diverse forms of chromosomes of the late prophase are successive morphological stages he argues this change of axial relations; and that might be justified if this premise were proven. But that it is not is shown by the evidence given by me (1901*a*, 1904) that certain chromosome pairs are characterized by certain forms in the spermatogonia as well as in the spermatocytes; a point which Baumgartner (1904) has recently corroborated and amplified.[1] Against this evidence McClung does not bring satisfactory proof that the differences in form express steps in axial changes. McClung's work appears to be very accurate, but I cannot follow him in this interpretation, and would ask the critical reader to compare his descriptions and figures with those on the related object given in the present paper. To prove his point he has to assume a complex axial metamorphosis, which is wholly unnecessary on the basis of a prereduction. The same criticism applies to the study of Gross (1904) on *Syromastes*, which is

[1] In the paper just mentioned Baumgartner claims that Sutton discovered a difference in size of the chromosomes, and states that he himself has "been fortunate enough to find a difference in form." I think I was the discoverer of both of these differences and expressed them distinctly for various forms, as Baumgartner will find stated in my papers of 1901 and 1904. But he deserves credit for distinguishing constant forms among chromosomes of the first spermatocytes.

the strongest argument yet given for postreduction; he is the solitary worker on the spermatogenesis of Hemiptera who has taken the postreductional view, and does it from a supposed secondary change in chromosomal form. Yet, strangely enough, he describes a prereductional division of the bivalent chromatin nucleolus, the only chromosome which is not said to pass through the stage of a cross! Its two whole univalent components become separated from each other in the first maturation mitosis. In his object the bivalent chromosomes are in some stages usually little longer than broad; they approach in some conditions more nearly the form of a cross than in any Hemipteron which I have studied. Had Gross taken up my old object, *Euchistus*, he would have found that X-shaped chromosomes do not occur at all, or only very rarely, that the phenomena there are accordingly simpler and more explicable than in *Syromastes*, and that intermediate forms between a chromosome elongated in one direction and one stretched out in another do not occur. Finally Gross admits that these forms admit of another interpretation: "Man könnte mir entgegenhalten, dass der von mir aus den Thatsachen erschlosene Modus der Tetradenbildung auf einer willkürlichen, durch nichts bewiesenen Annahme berühre. . . . . Sichere Anhaltspunkte dafür, nach welcher Richtung die Hälften der Kreuze aus einander weichen, lassen sich aus den beo. bachteten Figuren nicht entnehmen." I fully agree with him in this- But when he states, "Dasselbe gilt aber auch von der bis jetzt allgemein angenommenen Bildungsweise," he makes an error, for in some cases of spermatogenesis cross-shaped chromosomes do not occur, and that is so in *Euchistus*, and for such forms no voluntary assumptions are necessary. Gross' work appears very accurate, and I criticise only his interpretation of the crosses as intermediate forms. Evidently he is considerably influenced by Häcker's latest views. The same general criticism may be made of the work of Sutton (1902).

When we review all this work supposed to prove a postreduction, we find it based upon an incomplete series of stages, or upon forms with minute chromosomes of very diverse form, or upon such as have chromosomes in the form of rings and crosses. Every one will admit that chromosomes of such shapes are the most difficult to interpret: a tetrad with four parts of approximately equal size—where in it can we say lies the plane of the longitudinal split and where the line separating two univalent chromosomes? Just upon such chromosome forms is much of the postreduction argument based. The correct, because only decisive, method is not to reason from such forms, not to argue unnecessarily for a change in axis, but to explain such chromosome formation

from objects where the phenomena are simpler, where the chromosomes show a definite long axis in early stages, where the mode of formation of the bivalent chromosomes has been worked out, and where forms like rings and crosses do not occur. We must seek to explain the more complex from the more simple, not force an interpretation from the more complicated upon the more simple. The strongest argument for postreduction is that of McClung and Gross, and yet they are reasoning from the basis of perplexing rings and crosses. That such forms can be explained in quite a different manner, and their first division be regarded reductional instead of the second, I have shown for *Peripatus*, where the series of changes of the linin elements as well as of the chromatic are clearer than in any object yet seen by me.

To the idea of postreduction we can apply the criticism "not proven." No one can say that it does not occur, yet I do not hesitate to state as my opinion, coming from observations of some years upon a number of different animal forms, that it will be proved not to occur. And this is said with no intention of any disparity of the work of those who take the contrary stand, for they have accumulated very important and hard-won facts; it is only one of their interpretations that is being criticised. Prereduction is based upon a simpler reasoning and to some extent upon more patent phenomena.

So we reach the conclusion that maturation phenomena are all of the pseudomitotic type of Korschelt, and only of the prereductional kind. There is a mass of evidence for the view that in all cases the first maturation is the reductional one. Korschelt (1895) has described this for *Ophryotrocha*, Henking (1890) for *Pyrrhocoris*, Paulmier (1899) for *Anasa*, King (1901) for *Bufo*, Nichols (1902) for *Oniscus*, Lerat (1902) for copepods, Schockaert (1902) for *Thysanozoon*, Schreiner (1904) and Maréchal (1904) for fishes, McGill (1904) for *Anax*, Bouin and Collin (1901) for myriapods, and I for Hemiptera of different families (1898, 1899, 1901*a* and *b*), for *Peripatus* (1900), for salamanders (1903, 1904), and in the present paper for a grasshopper and a spider. And it will be noted that it is the most recent work which supports this view. Quite as conclusive evidence comes from an examination of the heterochromosomes, as we shall see later. Most of the recent work upon the botanical side corroborates this point of view, as that of Gregoire (1904), Rosenberg (1904), Strasburger (1904), Berghs (1904) and Farmer and Moore (1903).

From what I consider to be the strongest evidence available at the present time we find the following series of phenomena during the spermatogenesis of animals. There are a number of successive genera-

tions of spermatogonia, each with the normal number of univalent chromosomes (the heterochromosomes will not be considered in this place); all of their mitoses are equational. The last generation of them produces the spermatocytes of the first order. At an early period in these there takes place a pairing of the univalent chromosomes to form bivalent ones, which may be a junction end to end or side to side.[2] This is in each case a pairing of a paternal chromosome (one derived from the spermatid) with a maternal one (one from the ovotid). At an early stage of the growth period the bivalent chromosomes become more or less densely grouped, the synapsis stage, but the pairing of the chromosomes may commence shortly before this time. After this conjugation each univalent chromosome becomes longitudinally split, and no second splitting follows the first. There may or may not be a rest stage during the growth period, and when it occurs it may come before or after the synapsis stage. In the first maturation mitosis each bivalent chromosome undergoes a division in such a way that one whole univalent element passes into one daughter cell, the other one into the other cell; this is a true reduction division in the sense of Weismann, and accomplishes the reduction in number of the chromosomes; their conjugation in the rest stage had not effected reduction, but only the formation of pairs. The second maturation division is equational, along the line of the longitudinal split, so that the spermatid receives half the normal number, and each of them on comparison with those of the first spermatocytes is semivalent, but on account of their increase in size during the growth period virtually univalent. All the facts speak for a strict preservation through the whole germinal cycle of the individuality of the chromosomes.

From the correspondence determined by Henking (1890) and Hertwig (1890) between spermatogenesis and ovogenesis, by the one for insects and by the other for *Ascaris*, we might conclude that in all cases of ovogenesis also prereduction occurs, as indeed has been described for some animals. I think there is no sufficient evidence at present for doubting this conclusion, and much in favor of it. Yet it must be acknowledged that the ovogenetic processes are less easily analyzed,

[2] Maréchal is incorrect in stating that von Winiwarter (1900) first described this process; he simply reasoned that of three possible explanations of the origin of the bivalent chromosomes this was the most probable. Henking (1890), before von Winiwarter, had more conclusively argued for this, but did not see the first steps in the process. I was the first (1900) to describe all the steps in this series, and (1901*a*) to prove that each bivalent chromosome is formed by the conjugation of a paternal and a maternal one—this corroborated in the next year by Sutton (1902). Gross (1904) is of course in error when he calls this the "Häcker'sche Theorie." This important phenomenon is at last receiving rapid confirmation from many sides.

because of the larger growth period with its much greater degree of metabolism, which is responsible for a certain inclination, curiously enough still surviving in some minds, to doubt the individuality of the chromosomes. The cases of peculiar interest to the student of the germ cells are parthenogenetically developing eggs. All the investigators of parthenogenesis hold that both pole bodies represent equational divisions, or that the second is the reductional one; and very general is the opinion that the second maturation mitosis being reductional, and the lack of formation, or secondary retraction, of the second polar body being generally associated with normal parthenogenesis, it is effected that by parthenogenesis the number of chromosomes does not become halved. But there is no good ground for this view, and parthenogenesis with fertilization following in a subsequent generation is really better explained on the idea of a prereduction. For if the first maturation is reductional and the second (equational) one is eliminated, the parthenogenetic egg would have one-half the normal number of chromosomes; whether this number persists through all cell generations of the succeeding individual remains to be determined; there is some evidence that it may do so. If the half number does persist, then when an egg of the following individual becomes fertilized by a spermatozoon the normal number would be restored, instead of being multiplied one and a half times. This could not be effected if the second maturation mitosis were reductional, and the second polar body not produced. And of one point we can be reasonably certain: as Sutton (1903) has reasoned, there is no probability that in a reduction mitosis all the paternal chromosomes pass to one daughter cell and all the maternal chromosomes to another; in other words, there is no evidence that half the spermatids or ovotids contain only paternal elements and half only maternal. Indeed, the chance of this would decrease inversely in geometrical ratio with number of chromosomes. And therefore it is a wholly unfounded assumption to conclude, as some have done with greater ability in the construction of hypotheses than in reasoning from phenomena, that either or both pole bodies eliminate all the "male chromatin" (paternal chromosomes). The great weight of evidence is in favor of the view that the first maturation mitosis reduces the number of chromosomes, breaks apart the univalent components of the bivalent chromosomes, but does not do it in such a way as to separate all the paternal from all the maternal; and those who have founded hypotheses on contrary premises have been weaving ropes of sand.

4.—The Heterochromosomes.

These were discovered but not correctly interpreted by Henking (1890) and Wilcox (1895); first recognized as modified chromosomes by Paulmier (1899) and myself (1898); then described for a variety of Arthropoda by McClung (1899–1904), Sutton (1900, 1902), de Sinéty (1901), Wallace (1900), Gross (1904), McGill (1904), Baumgartner (1904), myself (1901*a* and *b*, 1904*a*), Voinov (1903) and Prowazek (1901); the last two papers I have not seen. In all these objects there occur in the spermatogenesis peculiarly modified chromosomes, which I have proposed (1904*a*) to include under the term "heterochromosomes." I had named them previously "chromatin nucleoli," though with full appreciation of their chromosomal nature, Paulmier "small chromosomes," McClung "accessory chromosomes," and de Sinéty "special chromosomes." Their essential characteristic is their difference in behavior from the other chromosomes in the growth period of the spermatocytes and ovocytes, as sometimes during the rest stages of the spermatogonia, a difference which appears usually to consist in the maintenance of their compact structure and deep-staining intensity, so that while the other chromosomes become long loops or even compose a reticulum, these do not undergo any such changes or only to slight extent. There is really not much known as yet of these modified chromosomes despite extended studies upon them, and at this place I wish mainly to draw attention to and try to explain differences in their behavior during the maturation mitoses, and so endeavor to explain certain phenomena that up to this time have been regarded as contradictory. They appear to be of very general occurrence in insects, have been found in spiders by Miss Wallace and by me (in the present paper), but so far seem not to be demonstrated for other objects. To be sure Blackman (1900) described an "accessory chromosome" in spermatocytes of *Scolopendra*, but did not describe its action in the spermatogonia nor even in the maturation mitoses, and has not proved in any manner that this body is not a true nucleolus; true nucleoli containing chromatin or even chromosomes are relatively rare in metazoan cells, but they sometimes occur (as, *e.g.*, I have shown for the ovocyte of *Paragordius* in a paper recently published), and what Blackman has described appears to be such a structure.

As I recently pointed out (1904*a*) there are two main kinds of heterochromosomes: such as occur in pairs in the spermatogonia and unite to form bivalent ones in the spermatocytes, which are the most frequent kind in the Hemiptera and were named "chromatin nucleoli" by me;

and such as are unpaired or single in the spermatogonia and so do not conjugate in the spermatocyte, which McClung calls "accessory chromosomes." Both these kinds agree essentially in their behavior during the growth period of the spermatocyte, and are clearly distinguishable from the other ("ordinary") chromosomes by their compact form and smooth outline; they differ with regard to the point of being single or double in the spermatogonia. Both kinds may occur in the same animal, as I have shown (1901*b*) for *Protenor*. In *Anasa* I found a pair of heterochromosomes in the ovogonia exactly like those in the spermatogonia, which suggests that the paired heterochromosomes will be found to occur in both maternal and paternal germ cells of the same species; but whether unpaired heterochromosomes occur in maternal germ cells is not known.

Heterochromosomes that are paired in the spermatogonia and unite to form bivalent ones in the spermatocytes I have described (1898, 1901*a*, 1901*b*, 1904*a*) for some forty species of Hemiptera, and in the present paper for *Lycosa* (a spider) and *Syrbula* (an Orthopteron); Henking (1890), Paulmier (1899) and Gross (1904) likewise for Hemiptera; and McGill (1904) for *Anax* (an Odonate). Heterochromosomes that are single in both spermatogonia and spermatocytes for *Orphania* and *Gryllus* by de Sinéty (1901), for *Protenor* by me (1901*a*), for *Xiphidium* by McClung (1902), *Brachystola* by Sutton (1900, 1902), and *Gryllus* by Baumgartner (1904).

Not to be confused with heterochromosomes are the "odd" chromosomes I described (1901*a*, *b*) for *Alydus*, *Harmostes* and *Œdancola*, chromosomes that seem to behave exactly like any ordinary chromosome during the growth period of the spermatocytes, and cannot be distinguished from them by any compactness of structure or intensity of stain, except that they do not form bivalent chromosomes by conjugation with others. I called them *odd* because in cases where they are present the spermatogonium has an *odd* or *uneven* number of ordinary chromosomes, and the odd one is that which does not have a homologous mate with which to pair during the synapsis stage. These resemble in certain respects the unpaired heterochromosomes, but differ in not maintaining a compact form during the growth period. These three genera of Hemiptera are the only known cases where there is an uneven number of chromosomes in the spermatogonia, without the odd chromosome being a heterochromosome.

And now we come to the point of the behavior of the heterochromosomes and the odd chromosomes during the maturation mitoses. With regard to the heterochromosomes (chromatin nucleoli) that occur

in pairs in the spermatogonia, I was able to determine the following relations (1901). In all the species of Hemiptera these unite to form one bivalent chromosome in the first spermatocytes, which appears clearly double at the time of the first maturation mitosis. In *Euchistus variolarius*, *Harmostes*, *Protenor* and *Œdancola* the heterochromosomes of this type divide reductionally in the first mitosis, so that their univalent components become separated; in the second mitosis each divides again, by comparison with the other chromosomes probably equationally, though I could not determine this in any decisive manner. The same process Gross (1904) has described for the chromatin nucleoli of *Syromastes*, and I have recently found it to hold for *Euchistus tristigmus*.[3] For *Anasa tristis*, *Alydus eurinus*, *Corizus*, *Oncopeltus*, *Calocoris*, *Acholla* and *Zaitha* I found (1901*a*) the bivalent heterochromosome to divide reductionally in the first mitosis, but did not determine its behavior in the second; this is also the case in *Lygus*, *Nobis*, *Corizus*, as I showed in the supplementary paper (1901*b*). Paulmier (1899) found the bivalent heterochromosome of *Anasa* to divide reductionally in the first mitosis, but not to divide in the second, in agreement with Henking's (1890) observations on *Pyrrhocoris*, and with those of McGill (1904) on *Anax*. That the bivalent heterochromosomes of *Syrbula* and *Lycosa* probably, but not certainly, divide first reductionally, then equationally, in the two maturation mitoses is shown in the present paper. Finally McClung (1900) describes for *Hippiscus* an accessory chromosome of the spermatocyte, said to divide in both maturation mitoses; he does not describe the relations for the spermatogonia, but it is quite probable to my mind that the phenomena in *Hippiscus* will be found essentially similar to those determined by me for *Syrbula*, namely, a bivalent heterochromosome in the first spermatocyte, formed by the conjugation of two univalent heterochromosomes of the spermatogonium.

We can summarize the facts of the preceding paragraph, noting parenthetically that for the details in the various species the reader must refer to the original descriptions, in the following statement: when heterochromosomes occur in pairs in the spermatogonia, *i.e.*, are of the type of "chromatin nucleoli," they always unite by conjuga-

[3] On a renewed study of my old preparations of the last species I find this to be certainly the case, and my earlier statement (1901*a*) was erroneous, to the effect that the bivalent heterochromosome divides first equationally, then reductionally. Also in my account of *E. variolarius* (1901*b*) I stated the heterochromosome in the second mitosis "is not always divided"; that must be amended to read "seems not always to be divided," in that in some of the spermatids it escapes detection by its small size or by being covered by another chromosome.

tion to form bivalent ones in the first spermatocytes, and all the describers except McClung agree that in the first maturation mitosis they always divide reductionally. No set of chromosomal structures is better adapted than such heterochromosomes to prove prereduction: there are two in the spermatogonium, which unite to form a bivalent one in the spermatocyte, and the separation of the univalent halves of the latter in the first mitosis is settled beyond any question of doubt for almost all the cases—for all the cases in which they can be recognized by peculiarities of form or size during this mitosis. Never in the spermatocytes do they take on the puzzling forms of rings and crosses which have misled so many good observers in the argument for postreduction. And it is significant that Gross (1904) shows the bivalent heterochromosome of *Syromastes* is prereductional in its division, and only by very indirect evidence attempts to show that the ordinary chromosomes divide postreductionally. As to the behavior of this kind of heterochromosome in the second maturation, for most of the species nothing positive could be decided; for other cases it has been shown that in some cases it divides in the second mitosis (probably equationally), as in *Euchistus, Harmostes, Protenor, Œdancola, Syromastes, Syrbula, Lycosa* and *Hippiscus,* while it does not divide in this second mitosis in *Anasa, Pyrrhocoris* and *Anax.*

Secondly, as to the division of the heterochromosomes that occur singly in the spermatogonia, and so undergo no conjugation in the spermatocytes. Those of *Orphania* (de Sinéty), *Gryllus* (de Sinéty and Baumgartner), *Brachystola* (Sutton), and *Xiphidium* (McClung do not divide in the first maturation mitosis, but do so in the second. Hence here again is prereduction: a whole chromosome passing undivided into one of the second spermatocytes, in the very mitosis which all these observers consider to be equational! The exceptional case is the unpaired heterochromosome of *Protenor* ("chromosome x"), which I described (1901*b*) as dividing transversely in the first mitosis, but not dividing in the second. I have recently gone over these old preparations with great care, and find nothing incorrect in my original description.

Thirdly, in regard to the divisions of the odd chromosomes of *Œdancola, Harmostes* and *Alydus*, which occur singly in the spermatogonia but are not heterochromosomes. In my original description (1901*b*) I did not determine their behavior positively in *Alydus* and *Harmostes*, beyond showing that they do not divide in one of the mitoses. I have recently studied them again, and find that in all these forms they divide in the first maturation mitosis but not in the second, just as is

the case with the unpaired heterochromosome of *Protenor* and what Gross (1904) has called the "accessory chromosome" in *Syromastes* (to which we shall recur). They do not *appear* bivalent in the first spermatocytes; and whether their division in the first maturation mitosis is transverse or parallel to their long axis was not determined on account of their nearly spherical form.

Now to him who has had the patience to follow this account, which gives only a brief statement of some of the results of previously detailed observations, the occurrence and behavior of the two kinds of heterochromosomes and of the odd ordinary chromosomes may well seem difficult to reconcile. But there is nevertheless a general conformity of process here, which has not been elucidated heretofore. Whenever the heterochromosomes occur in pairs in the spermatogonia they always conjugate to form bivalent ones in the first spermatocytes, and their univalent components become separated in the first maturation mitosis, *i.e.*, divide prereductionally. This is strictly in confirmation with the doctrine we have tried to lay down in this paper, that the separation of entire univalent chromosomes, *i.e.*, their reduction in number, is always accomplished in the first mitosis. At the same time we have to bear in mind that there is no evidence that chromosomes divide in different ways in the first maturation mitosis, some equationally and some reductionally; it is very probable that does not happen, and indeed until proof is brought to the contrary we are justified in maintaining that it does not occur. This is an important premise in interpreting the divisions of the heterochromosomes and ordinary chromosomes that occur singly in the spermatogonia. Now in the Orthoptera (*Orphania, Gryllus, Xiphidium, Brachystola*) the heterochromosome is single in the spermatogonia; single, therefore, in the spermatocytes, it does not divide in the first maturation mitosis, but does in the second. Because it does not divide in the first mitosis it must be either univalent or else already in the spermatogonia be composed of two so firmly united that they cannot be divided in the reduction mitosis; its division in the second mitosis must be equational, and all the descriptions show this to be so. Now in *Protenor* the case is reversed; the single heterochromosome divides in the first mitosis, but not in the second, exactly like the odd ordinary chromosomes of the Hemiptera, but apparently the reverse of the single heterochromosomes of Orthoptera. Since this heterochromosome of *Protenor* and the odd ordinary chromosomes of three other Hemipteran species divide during the reduction mitosis, these chromosomes must be already bivalent within the spermatogonium—the single one there be

two in close union, but not so close as to prevent their separation in the reduction mitosis. There is some observational proof for this, in that the odd chromosome or unpaired heterochromosome in the spermatogonium sometimes exhibits a transverse constriction, as if marking the point where two had joined, in *Harmostes* and *Protenor;* and in *Protenor* the division of the heterochromosome in the reduction mitosis is at right angles to its long axis. The failure to divide in the second mitosis can only be ascribed to an incomplete process of longitudinal splitting during the growth period. We can thus express the likeness and difference between the single heterochromosomes and odd ordinary chromosomes of the Hemiptera and the single heterochromosomes of the Orthoptera; they all agree in dividing reductionally in the first maturation mitosis, whether by a separation of two univalent components or by a transport of the whole chromosome into one of the daughter cells; they differ merely in not undergoing or in undergoing an equational splitting in the second mitosis. We can sum this up in the statement: all chromosomes and heterochromosomes, be they paired or single in the spermatogonia, divide reductionally in the first maturation mitosis, whether this division consist in two univalent components separating from each other or a single component passing undivided into one of the second spermatocytes.

And now we come to another point with regard to a general uniformity of heterochromosomes. I first showed (1901*a*, *b*) that the ordinary chromosomes in the spermatogonia are arranged in pairs, so that, *e.g.*, fourteen chromosomes form seven pairs, the two of a pair being alike in size; and I showed for several species that whenever spermatogonial chromosomes show marked differences in size they can be recognized again in the bivalent chromosomes of the spermatocytes. Sutton (1902) corroborated this for *Brachystola.*[4] And later I showed (1904*a*) corresponding chromosomes in the spermatogonia are alike not only in size but also in form. We have just seen, also, that one kind of heterochromosomes, the chromatin nucleoli, occur in pairs in the spermatogonia—where there is one bivalent one of these in the spermatocytes it corresponds to two in the spermatogonia. Further than this, we have shown that the odd ordinary chromosomes of Hemiptera and the unpaired heterochromosomes of *Protenor* must be regarded as already

[4] Boveri (1904), in his recent review, ascribes the main credit of this discovery to Sutton (1902), as others have done; but the point was very clearly stated in my papers, illustrated on a number of species, and, furthermore, I demonstrated that chromosomes of corresponding size conjugate to form the bivalent ones. Quite a number of papers have come out recently with "new discoveries" which had already been made in my papers on *Peripatus* and Hemiptera.

bivalent in the spermatogonium—there as a chromosome pair with the components closely united instead of being, as with most of the chromosomes, separated. Can we go further than this, and consider the unpaired heterochromosomes of the Orthoptera to be also already bivalent in the spermatogonium, but with the univalent parts so closely united that they do not become separated even in the reduction mitosis? The heterochromosomes of the Orthoptera appear to be usually larger than the ordinary chromosomes, which is the only observational evidence for the idea that they may have the value of more than one chromosome, and sometimes they are much larger. Such evidence is, of course, not at all sufficient. But should they be ultimately proven to be bivalent in the spermatogonia, a further uniformity would evince itself: all heterochromosomes and all ordinary chromosomes would be paired in the spermatogonia, whether the two members of a pair be separated there (univalent) or be united (bivalent); in the former case they would become bivalent by conjugation for the first time in the spermatocytes, in the second case they would pass over already bivalent to the spermatocytes. In any event an even number of univalent chromosomes in the spermatogonia and half that number of bivalent ones in the spermatocytes would be the primitive (unmodified) condition, as it is the one most usually found. In the preceding paragraph it was shown to be probable that the odd ordinary chromosomes of the Hemiptera and the unpaired heterochromosome of *Protenor* are already bivalent in the spermatogonia; this may or may not be the case with the unpaired heterochromosomes of the Orthoptera, but if it is the case, as I think is somewhat probable, then the following conclusion is reached—a conclusion well based at least for the odd ordinary chromosomes and the unpaired heterochromosome of *Protenor:* heterochromosomes that are paired in the spermatogonia and become bivalent in the spermatocytes would be an earlier condition, and would lead to the later condition of heterochromosomes unpaired in the spermatogonia by conjugation of their univalent components in spermatogonic cell generations. In this way unpaired heterochromosomes would be later modifications of the paired; and in the same manner, unpaired ordinary chromosomes later modifications of paired ordinary chromosomes. Two univalent chromosomes of a spermatogonium might conjugate to form one bivalent one before the spermatocyte stage, this would then be an odd ordinary chromosome, which later might or might not become an unpaired heterochromosome; or two ordinary chromosomes of a spermatogonium might become heterochromosomes (chromatin nucleoli) but still remain univalent in this cell (conjugating not before the

spermatocyte stage), and two such univalent heterochromosomes might or might not later conjugate in a spermatogonium to form an unpaired heterochromosome. On such a premise paired heterochromosomes and chromosomes within the spermatogonia would be an earlier condition than unpaired ones, and unpaired heterochromosomes could be formed in two ways.

The conclusions of the preceding paragraph are put forward merely as tentative suggestions, and in no sense as final conclusions; the phenomena are too complex as yet for any thorough analysis and interpretation. But amongst all this complexity a certain agreement in the phenomena becomes evident, and this it is our business to discover. I still see no reason, despite the criticisms of McClung, to modify my original standpoint (1901*b*), that there is a transmutation in chromosomal numbers just as in any other parts of the organization, and that the heterochromosomes are chromosomes on the way to disappearance; following Paulmier's (1899) earlier contention that they are degenerated chromosomes. McClung (1900, 1902) urges that they are frequently larger than other chromosomes and show just as many signs of active metabolism. But neither Paulmier nor I regarded them as dead structures; and I pointed out that they seem to have a different metabolic energy from the ordinary chromosomes, because in some species of Hemiptera they are regularly attached to the true nucleolus, which condition the other chromosomes do not share, and have a different position within the nucleus (almost always against its membrane). There can well be no question that they are metabolically different, else they would not behave so differently, with a peculiar autonomy. McClung has described them only for Orthoptera, where they are frequently the largest chromosomes. But the paired heterochromosomes of the Hemiptera are usually the smallest of all, sometimes very minute granules (as in *Peribalus, Cœnus, Trichopepla, Corizus, Coriscus, Prionidus*); and when there are several pairs within a cell, as, *e.g., Acholla,* all of them are smaller than the other chromosomes. So I considered them degenerate in the sense that they no longer carry on exactly the same activities as the ordinary chromosomes, from which they must be derived, but have taken on other energies and have in most of the described cases become smaller. The excessively minute heterochromosomes would then be the last perceptible stage in their history; for surely there is no reason to consider this the first stage—to consider them as orginating as buds from larger ordinary chromosomes. Unpaired heterochromosomes do not conjugate during the growth period, for the reason of the absence of a mate with which to unite; and in

cases of bastardization between different species, as described by Guyer (1902) and Moenkhaus (1904), the maternal and paternal chromosomes fail to conjugate. Or, if the parents have different numbers of chromosomes, some of those of the parent with the larger species are forced to remain univalent during the growth period, as shown by Rosenberg (1903) for *Drosera*. Facts like these might suggest that the presence of heterochromosomes has been produced by bastardization of species with different number of chromosomes. But that could be the case only of unpaired heterochromosomes; it would not explain the paired ones, and we have found that the unpaired kind are probably derivable from the paired. Again, they have been found in all insects in which they have been sought for, or in nearly all, but it would be rash to conclude that all these species of insects have arisen as bastards between parental forms with different chromosomal numbers. Therefore there is no good reason to refer the heterochromosomes to any hybridization process; and every reason to consider them as modified conditions of the ordinary chromosomes, formed in some cases concomitantly with a change in chromosomal number, probably from a higher number to a lower, chromosomes with a different metabolic activity and on the way to disappearance. A remarkable fact, for which I see no explanation whatsoever, is their very general occurrence among insects, and their absence elsewhere except in spiders; but they may be found in other groups when the attention is given them that they deserve. McClung (1902*a*) has put out the hypothesis that they are sex-determinants, reasoning from the condition of the unpaired heterochromosome of *Xiphidium;* here only half of the spermatids receive the division products, and he argues that its presence in them may determine the male sex. This is only a hypothesis, and as yet we do not even know whether in the ovocytes of such species similar heterochromosomes may not occur. Indeed, whether spermatozoa with and those without heterochromosomes are equally capable of fertilization is not known, and would be exceedingly difficult to determine. Further, in some species of Hemiptera all the spermatozoa receive division products of the heterochromosomes, and on McClung's hypothesis all spermatozoa in such species would produce males.

On the question of the perpetuation from generation to generation of an odd number of heterochromosomes or ordinary chromosomes I have touched at another place (1901*b*); but now I am convinced it is inutile to discuss this problem until we have facts of their behavior in the maternal germ cells.

In conclusion, attention should be drawn to the recent divergent

ideas of Gross (1904). He describes for *Syromastes*, a Hemipteron, two pairs of modified chromosomes: one pair of these, which he calls *chromatin nucleoli*, differ from the other chromosomes in acting like heterochromosomes during the growth period of the spermatocytes, but agree with them in dividing in both maturation mitoses; the other pair, which he calls *accessory chromosomes*, differ from the ordinary chromosomes in not dividing during the second maturation mitosis, but behave exactly like them during the growth period. His chromatin nucleoli, which are not recognizable until the stage of the spermatocytes, are said not to differ in volume from the ordinary chromosomes in the spermatogonia; while his accessory chromosomes are described as the smallest of all the chromatin elements. Both kinds of these bodies are paired and univalent in the spermatogonia, and by conjugation become bivalent in the spermatocytes. Now Gross reasons these are separate genealogical conditions of one and the same structure. He argues that a pair of unmodified ordinary chromosomes of the spermatogonium become in the spermatocytes chromatin nucleoli, which there act like heterochromosomes, preserve their compact structure and undergo no longitudinal split, and divide in both maturation mitoses, so that each spermatid receives a half of each univalent component. A spermatozoon formed from such a spermatid unites with an ovotid with a corresponding semivalent chromatin nucleolus. But instead of these two semivalent heterochromosomes (chromatin nucleoli) of the fertilized egg appearing in the next following generation of spermatogonia as chromatin nucleoli, he conceives them to appear in the form of the pair of small accessory chromosomes, which form a bivalent one in the following spermatocyte, divide in the first maturation mitosis but not in the second, so that half of the spermatids receive a half of each of them. So he interprets them both as chromosomal elements whose maturation divisions are continued over two generations of individuals; although he really describes three divisions of them, two for the chromatin nucleoli and one for the accessory chromosomes. We need not enter here upon his further deductions from this interpretation, but shall consider simply its probability. A strong objection that suggests itself is this: all the individuals studied by him showed in the spermatocytes two chromatin nucleoli and two accessory chromosomes; but this would be impossible if in every other generation the chromatin nucleoli changed into accessory chromosomes, for then one should find in the cells of some individuals no chromatin nucleoli but four accessory chromosomes. And if, and Gross suggests this possibility, from time to time successive pairs of ordinary chromosomes become chromatin nucleoli,

then in the course of time all the chromosomes would become chromatin nucleoli; yet in no individuals were found more than one pair. So from whatever standpoint we regard his explanation its improbability becomes manifest. On the other hand his chromatin nucleoli behave exactly like the chromatin nucleoli (paired heterochromosomes) of *Euchistus*, except that they are not distinguishable in the spermatogonia (in some other Hemiptera they are also not recognizable in these cells); so there is every reason to consider them as persisting from individual to individual as chromatin nucleoli. What he calls in *Syromastes* the accessory chromosomes are not heterochromosomes at all, so certainly not later stages at all of chromatin nucleoli, for he describes them as conducting themselves exactly like the ordinary chromosomes during the growth period; the bivalent accessory chromosome of the spermatocytes differs only from the other bivalent chromosomes in failing to divide in the second mitosis. I think this "accessory chromosome" of *Syromastes* is to be considered a stage leading to that of the unpaired heterochromosome of *Protenor;* they resemble each other in failing to divide in the second maturation mitosis, and though the one in *Protenor* is virtually single in the spermatogonia we have given reasons to show that it is probably bivalent there. The failure to divide in the second mitosis can for both be ascribed to incompleteness of the longitudinal split. And this is surely a far simpler interpretation of the phenomena in *Syromastes*, one much more in accordance with what has been described in other objects, than that elaborated by Gross. It is hardly necessary to adjoin that such a process as the two maturation divisions of one pair of chromosomes being continued over two germinal cycles has no known counterpart in other animals, and so needs the most rigid observational demonstration.

Gérard (1901) has described for *Prosthecerœus* and Schockaert (1901) for *Thysanozoon* a peculiar deep-staining thread within the ovocytes which divides into two, and is said to give rise to the egg centrosomes; it is for future research to determine whether this structure may have any relation to the heterochromosomes.

Literature.

Baumgartner, W. J. 1904. Some New Evidences for the Individuality of the Chromosomes. *Biol. Bull.*, 8.

Berghs, J. 1904*a*. La formation des Chromosomes hétérotypiques dans la sporogénèse végétale. 2. *La Cellule*, 21.

—— 1904*b*. *Idem*. 3. *Ibid.*

Blackman, M. W. 1901. Spermatogenesis of the Myriapods. *Bull. Univ. Kansas*, 10.

Bouin, P., and Collin, R. 1901. Contribution à l'étude de la division cellulaire chez les Myriapodes. *Anat. Anz.*, 20.

Boveri, T. 1887. Zellen-Studien. 1. Die Bildung der Richtungskörper bei Ascaris megalocephala and Ascaris lumbricoides. Jena.

—— 1904. Ergebnisse über die Konstitution der chromatischen Sustanz des Zellkerns. Jena.

Brauer, A. Zur Kenntniss der Spermatogenese von Ascaris megalocephala. *Arch. mikr. Anat.*, 42.

Carnoy, J. B. 1885. La Cytodiérèse chez les Arthropodes. *La Cellule.*

Farmer, J. B., and Moore, J. E. S. 1903. New Investigations into the Reduction Phenomena of Animals and Plants. *Proc. Roy. Soc. London*, 72.

Francotte, P. 1898. Recherches sur la maturation, la fécondation et la segmentation chez les Polyclades. *Arch. Zool. expér. gén.*

Fol, H. 1891. Le Quadrille des centres. Un épisode nouveau dans l'histoire de la fécondation. *Arch. sci. phys. math.*

Gérard, O. 1901. L'ovocyte du premier ordre du Prostheceræus vittatus. *La Cellule*, 18.

Grégoire, V. 1904. La réduction numérique de chromosomes et les cinèses de maturation. *La Cellule*, 21.

Griffin, B. B. 1899. Studies on the Maturation, Fertilization and Cleavage of Thalassema and Zirphæa. *Journ. Morph.*, 15.

Gross, J. 1904. Die Spermatogenese von Syromastes marginatus L. *Zool. Jahrb.*, 20.

Guyer, M. F. 1900 (really published two or three years later). Spermatogenesis of Normal and of Hybrid Pigeons. Chicago.

Häcker, V. 1891. Die Richtungskörperbildung bei Cyclops und Canthocamptus. *Ber. naturf. Ges. Freiburg*, 6.

—— 1892. Die Eibildung bei Cyclops und Canthocamptus. *Zool. Jahrb.*, 5.

—— 1893. Das Keimbläschen, seine Elemente und Lageveränderungen. *Arch. mikr. Anat.*, 41.

—— 1895. Ueber die Selbständigkeit der väterlichen und mütterlichen Kernsubstanz während der Embryonalentwickelung von Cyclops. *Ibid.*, 46.

—— 1899. Praxis und Theorie der Zellen- und Befruchtungslehre. Jena.

—— 1902. Ueber das Schicksal der elterlichen und grosselterlichen Kernanteile, Jena. *Jenaische Zeitschr. Naturw.*, 37.

—— 1904*a*. Bastardirung und Geschlechtszellenbildung. Ein kritisches Referat. *Zool. Jahrb.*, Suppl., 7.

—— 1904*b*. Heterotypische Teilung, Reduktion und andere zelltheoretische Begriffe. *Zool. Anz.*, 28.

Henking, H. 1890. Ueber Spermatogenese und deren Beziehung zur Eientwicklung bei Pyrrhocoris apterus M. *Zeit. wiss. Zool.*, 51.

Hertwig, O. 1890. Vergleich der Ei- und Samenbildung bei Nematoden. *Arch. mikr. Anat.*, 36.

King, H. D. 1901. The Maturation and Fertilization of the Egg of Bufo lentiginosus. *Journ. Morph*, 17.

Klinckowström, A. v. 1897. Beiträge zur Kenntniss der Eireifung und Befruchtung bei Prostheceræus. *Arch. mikr. Anat.*, 48.

Korschelt, E. 1895. Ueber Kerntheilung und Befruchtung bei Ophryotrocha puerilis. *Zeit. wiss. Zool.*, 60.

Korschelt, E., and Heider, K. 1903. Lehrbuch der vergleichenden Entwick-

lungsgeschichte der wirbellosen Thiere. Allgemeiner Theil, Zweite Lieferung, Jena.

Lerat, P. 1902. La première cinèse de maturation dans l'ovogénèse et la spermatogénèse du Cyclops strenuus. *Anat. Anz.*, 21.

Linville, H. R. 1900. Maturation and Fertilization in Pulmonate Gasteropods. *Bull. Mus. Comp. Zool. Harvard Univ.*, 35.

McClung, C. E. 1899. A Peculiar Nuclear Element in the Male Reproductive Cells of Insects. *Zool. Bull.*, 2.

—— 1900. The Spermatocyte Divisions of the Acrididæ. *Bull. Univ. Kansas*, 9.

—— 1901. Notes on the Accessory Chromosome. *Anat. Anz.*, 20.

—— 1902*a*. The Accessory Chromosome—Sex Determinant? *Biol. Bull.*, 3.

—— 1902*b*. The Spermatocyte Divisions of the Locustidæ. *Kansas Univ. Sci. Bull.*, 1.

McGill, C. 1904. The Spermatogenesis of Anax junius. *Univ. Missouri Studies*, 2.

Maréchal, J. 1904. Ueber die morphologische Entwickelung der Chromosomen im Keimbläschen des Selachiereies. *Anat. Anz.*, 25.

Moenkhaus, W. J. 1904. The Development of the Hybrids between Fundulus heteroclitus and Menidia notata, with especial Reference to the Behavior of the Maternal and Paternal Chromatin. *Amer. Journ. Anat.*, 3.

Montgomery, T. H., Jr., 1898. The Spermatogenesis in Pentatoma up to the Formation of the Spermatid. *Zool. Jahrb.*, 12.

—— 1899. Chromatin Reduction in the Hemiptera: a Correction. *Zool. Anz.*, 22.

—— 1900. The Spermatogenesis of Peripatus (Peripatopsis) balfouri up to the Formation of the Spermatid. *Zool. Jahrb.*, 14.

—— 1901*a*. A Study of the Chromosomes of the Germ Cells of the Metazoa. *Trans. Amer. Phil. Soc.*, 20.

—— 1901*b*. Further Studies on the Chromosomes of the Hemiptera heteroptera. *Proc. Acad. Nat. Sci. Philadelphia.*

—— 1903. The Heterotypic Maturation Mitosis in Amphibia and its General Significance. *Biol. Bull.*, 4.

—— 1904*a*. Some Observations and Considerations upon the Maturation Phenomena of the Germ Cells. *Ibid.*, 6.

—— 1904*b*. Prof. Valentin Häcker's Critical Review on Bastardization and Formation of the Sex Cells. *Zool. Anz.*, 27.

Nichols, M. L. 1902. The Spermatogenesis of Oniscus asellus Linn., with especial Reference to the History of the Chromatin. *Proc. Amer. Phil. Soc.*, 41.

Paulmier, F. C. 1899. The Spermatogenesis of Anasa tristis. *Journ. Morph.*, 15, Supplement.

Prowazek, S. 1901. Spermatogenese der Weinbergschnecke. *Arb. zool. Inst. Wien*, 13.

—— 1902. Ein Beitrag zur Krebsspermatogenese. *Zeit. wiss. Zool.*, 71.

Rath, O. Vom. 1892. Zur Kenntniss der Spermatogenese von Gryllotalpa vulgaris Latr. *Arch. mikr. Anat.*, 40.

—— 1895. Neue Beiträge zur Frage der Chromatinreduction in der Samen- und Eireife. *Ibid.*, 46.

Rosenberg, O. 1903. Das Verhalten der Chromosomen in einer hybriden Pflanze. *Ber. Deutsch. Bot. Ges.*, 21.

—— 1904*a*. Ueber die Tetradenteilung eines Drosera-Bastardes. *Ibid.*, 22.

—— 1904*b*. Ueber die Reduktionsteilung in Drosera. *Meddel. Stockholms Hogsk. Bot. Inst.*

Rückert, J. 1894. Zur Eireifung bei Copepoden. *Anat. Hefte.*

Sabaschnikoff, M. 1897. Beiträge zur Kenntniss der Chromatinreduktion in der Ovogenese von Ascaris megalocephala bivalens. *Bull. Soc. imp. Natur. Moscou.*

Schockaert, R. 1901. L'ovogénèse chez le Thysanozoon brocchi. Première Partie. *La Cellule*, 18.

—— 1902. L'ovogénèse chez le Thysanozoon brocchi. Deuxième Partie. *La Cellule*, 20.

Schreiner, A. and K. E. 1904. Die Reifungsteilungen bei den Wirbeltieren. Ein Beiträg zur Frage nach der Chromatinreduktion. *Anat. Anz.*, 24.
Sinéty, R. de. 1901. Recherches sur la Biologie et l'Anatomie des Phasmes. *La Cellule.*
Strasburger, E. 1904. Ueber Reduktionsteilung. *Sitzb. preuss. Akad. Wiss. Berlin.*
Stricht, O. Van der. 1898. La formation des deux globules polaires et l'apparition des spermocentres dans l'oeuf de Thysanozoon Brocchi. *Arch. de Biol.*, 15.
Sutton, W. S. 1900. The Spermatogonial Divisions in Brachystola magna. *Bull. Univ. Kansas*, 9.
—— 1902. On the Morphology of the Chromosome Group in Brachystola magna. *Biol. Bull.*, 4.
—— 1903. The Chromosomes in Heredity. *Ibid.*, 4.
Voinov, D. N. 1903. La spermatogénèse d'été chez le Cybistes roeselii. *Arch. zool. expér. gén.* (4), 5.
Wagner, J. 1896. Einige Beobachtungen über die Spermatogenese bei den Spinnen (Vorläufige Mittheilung). *Zool. Anz.*, 19.
Wallace, L. B. 1900. The Accessory Chromosome in the Spider. *Anat. Anz.*, 18.
Wilcox, E. V. 1895. Spermatogenesis of Caloptenus femur-rubrum and Cicada tibicen. *Bull. Mus. Com. Zool. Harvard Univ.*, 27.
Winiwarter, H. von. 1900. Recherches sur l'Ovogénèse et l'Organogénèse de l'Ovaire des Mammifères (Lapin et Homme). *Arch. de Biol.*, 17.

Explanation of Plates IX and X.

All the figures were drawn by the author with the aid of the camera lucida, at a magnification of about 1800 diameters. In all N. 2 denotes the heterochromosome.

Plate IX. *Syrbula acuticornis* Bruner.

Fig. 1.—Spermatogonium, rest stage.
Figs. 2–6.—Spermatogonia, successive prophases.
Figs. 7, 8.—Spermatogonia, pole views of the monaster stage.
Fig. 9.—Lateral view of preceding stage.
Fig. 10.—Lateral view of spermatogonic anaphase.
Fig. 11.—Pole view of first spermatocyte, showing all the chromosomes, shortly after preceding stage.
Figs. 13–15.—First spermatocytes, successive early stages; in 15 only the nucleus shown.
Fig. 16.—Rest stage of first spermatocyte.
Figs. 17, 18.—Successive stages immediately following the preceding.
Figs. 19–22.—Successive stages of the synapsis, all lateral views, 19 showing only the nucleus.
Figs. 23–25.—Nuclei in post-synapsis stage.
Fig. 26.—Bivalent heterochromosome of this stage, consisting of two closely apposed univalent members, of which the upper one is stippled.
Figs. 27–31.—Successive prophases of first maturation mitosis; in the first three only the nucleus shown.
Figs. 32, 33.—Lateral views of the first maturation spindle; fig. 33 seen obliquely so that only one spindle pole shows.

Plate X, Figs. 34–40.—*Syrbula acuticornis* (continuation).

Figs. 34–36.—Successive anaphases of first maturation mitosis; in 36 the largest chromosome of the upper plate is longitudinally split, but so that one split half covers the other.
Fig. 37.—Oblique lateral view of one daughter chromosome plate, anaphase of the same mitosis.
Fig. 38.—Pole view of chromosome plate of the second spermatocyte.
Fig. 39.—Lateral view of second maturation spindle.

Fig. 40.—Pole view of chromosome plate of the spermatid.
Figs. 41–65.—*Lycosa insopita* Montg.
Fig. 41.—Pole view of spermatogonium, monaster stage.
Figs. 42–44.—Lateral views of synapsis stages.
Fig. 44*bis*.—*A* and *B* each represent a bivalent and longitudinally split heterochromosome of the synapsis stage, and the curved line near each an arc of the nuclear membrane.
Figs. 45, 46.—Lateral views of nuclei, postsynapsis.
Figs. 47–49.—Nuclei in early prophases of first maturation mitosis.
Figs. 50–52.—Nuclei in later prophases.
Figs. 53, 54.—Pole views of monaster, first maturation mitosis.
Figs. 55–58.—Lateral views of the same stage.
Fig. 59.—Anaphase of first maturation mitosis.
Figs. 60–63.—Pole views of the chromosome plates of second spermatocytes.
Figs. 64, 65.—Lateral views of second maturation spindle; 65 oblique so as to show only one spindle pole.

Postscript.

Some time after the preceding was sent to press the following papers were received, all confirmatory of my views upon chromosomal conjugation and reduction: L. B. Wallace, "The Spermatogenesis of the Spider," *Biol. Bull*, 8, 1905; L. T. Dublin, "The History of the Germ Cells in Pedicellina americana," *Ann. New York Acad. Sci.*, 16, 1905; and J. B. Farmer and J. E. S. Moore, "On the Maiotic Phase (Reduction Divisions) in Animals and Plants," *Quart. Jour. Micr. Sci.*, 48, 1905. Dublin's paper is of particular importance, because he finds perfect agreement in both ovogenesis and spermatogenesis. Miss Wallace finds that the accessory chromosomes do not divide in either maturation mitosis; and believes that only that fourth of the spermatids which receives them become functional spermatozoa, so that the remaining three-fourths "are regarded as homologous to the polar bodies thrown off by the ovum."